2958-Pos Board B388

M1 and M2 Microglia Exhibit Significant Differences in their K^+ Channel Expression

Eva Melanie Grossinger[1], Hai Minh Nguyen[1], Yi-Je Chen[1], Izumi Maezawa[2], Heike Wulff[1].
[1]Pharmacology, University of California Davis, Davis, CA, USA, [2]Department of Pathology and Laboratory Medicine, University of California Davis, Sacramento, CA, USA.

Microglia effector functions are widely associated with specific phenotypes, which are referred to as M1 and M2. Classically activated M1 microglia release pro-inflammatory cytokines and neurotoxic molecules and have been associated with neurological damage in ischemic stroke and Alzheimer's disease. Alternatively activated M2 microglia exhibit beneficial immunological effector functions such as phagocytosis of debris and release of anti-inflammatory and neurotrophic factors. Similar to B- and T-cells, microglia activity is regulated by calcium (Ca^{2+})-signaling, which is maintained by potassium (K^+) channels. We here investigated whether M1 and M2 microglia differ in their K^+ channel expression by differentiating neonatal mouse microglia into M1 and M2 phenotypes using lipopolysaccharide (LPS) and/or interferon-gamma or IL-4 and studying the cells by whole-cell patch-clamp. We identified three types of K^+ channels based on their biophysical and pharmacological fingerprints: a use-dependent, outwardly rectifying current sensitive to the Kv1.3 blockers PAP-1 and ShK-L5, an inwardly rectifying Ba^{2+}-sensitive Kir2.1 current, and a Ca^{2+}-activated, TRAM-34-sensitive KCa3.1 current. M1 microglia, obtained by stimulation with LPS or a combination of LPS and interferon-gamma exhibited high Kv1.3 current densities (~ 30-60 pA/pF at 40 mV) and virtually no KCa3.1 and Kir currents, while IL-4 stimulated M2 microglia exhibited large Kir currents (~ 10 pA/pF at −120 mV). KCa3.1 currents were generally low but moderately increased following stimulation with interferon-gamma or ATP. This differential K^+ channel expression pattern suggests that K^+ channel modulators could be used to selectively inhibit detrimental neuroinflammatory functions of microglia.

This work was supported by RO1 GM076063 and RO1 AG043788A1 *from the NIH.*

2959-Pos Board B389

Atherogenic Very-Low-Density Lipoprotein Shortens Atrial Action Potential Duration by Increasing Potassium Currents and Calcium Transient

Hsiang-Chun Lee[1,2], Chi Wei[3], Liang-Yin Ke[3], Pei-Shang Tsai[3], Hsin-Ting Lin[3], Yi-Lin Shiao[3], Bin-Nan Wu[4], Chu-Huang Chen[3,5], Sheng-Hsiung Sheu[1,2].
[1]Division of Cardiology, Department of Internal Medicine, Kaohsiung Medical University Hospital, KMU, Kaohsiung, Taiwan, [2]Internal Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [3]Center of Lipid Biosciences, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [4]Department of Pharmacology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [5]Vascular & Medicinal Research, Texas Heart Institute, Houston, TX, USA.

Background: Compared to that of healthy normal subjects, plasma very low density lipoprotein (VLDL) of patients with the metabolic syndrome (MetS) has been shown to be more electronegative and atherogenic. Given the association between MetS and increased prevalence of atrial fibrillation (AF), we investigated the mechanistic role of VLDL in the AF pathogenesis.

Methods: We extracted VLDL via peripheral blood obtained from normal, healthy volunteers and MetS individuals. The normal-VLDL and MetS-VLDL samples were treated to HL-1 atrial cardiomyocytes respectively for 12 hours before experiments. Whole-cell patch clamp was used for recording the action potentials, voltage-gated potassium currents, and L-type calcium currents. Calcium image with Fura-2-AM Ca^{2+} indicator was applied for the intracellular calcium measurements.

Results: MetS-VLDL treated HL-1 cells exhibited significantly higher densities of repolarizing potassium currents, IKs and IKr. MetS-VLDL shifted the activation curve of ICaL toward more negative membrane potentials. Intracellular calcium signals were significantly enhanced by MetS-VLDL but not by normal-VLDL. MetS-VLDL significantly shortened action potential durations (MetS-VLDL 178.1 ± 32.0 msec vs control 257.2 ± 52.7 msec; P=0.0017). Addiotnally, frequent occurrences of early after-depolarization on action potentials were noted in MetS-VLDL treated HL-1 cells.

Conclusions: The VLDL of MetS individuals augmented repolarizing potassium currents, increased intracellular calcium release, and significantly shortened action potentials. These changes may contribute in coordination to increased AF vulnerability in MetS.

2960-Pos Board B390

New Insight into the Involvement of Large-Conductance Calcium-Activated-Potassium-Channel(BK) in Cell Viability: Pathophysiological Implications in Neuromuscular Disorders

Angela Curci, Antonietta Mele, Giulia Maria Camerino, Diana Conte, Domenico Tricarico.
Dept. of Pharmacy-Drug Science, University of Bari, Bari, Italy.

The large conductance Ca^{2+}-activated K^+-channel (BK) is involved in several pathophysiological conditions including periodic paralysis (PP) and myotonia. Acetazolamide (ACTZ) a carbonic anhydrase inhibitor used in these conditions, acts targeting BK in PP. Here we investigated the involvement of BK channel in neuronal viability (SH-SY5Y cell) by combining patch-clamp technique and cell proliferation assays. We performed these measurements in the presence or absence of the selective BK channel blocker Iberiotoxin (IbTX) (10-400x10^{-9}M), the unselective K^+-channels blocker Tetraethylammonium (TEA) (0.01-1x10^{-3}M), and the BK channel openers NS1619 (10-100x10^{-5}M) and ACTZ (0.1-200x10^{-6}M). Patch-clamp recordings showed that at +30mV (Vm) IbTX and TEA reduced whole cell K^+-current in a concentration -dependent manner with an Imax of −46% and −90% respectively. NS1619 enhanced K^+-current of +141% at −10mV (Vm). Acetazolamide, that in muscle acts as a BK opener, in neurons caused a concentration-dependent block of K^+-current at +30mV(Vm) with an IC50 of 1.73x10^{-7}M an Imax of −40% (slope=0.37) (Number of patches=12). These drugs exert their effects also on neuronal cell viability, enhancing it: IbTX showed a maximal proliferative effect (MPE) of +46% at 10^{-8}M concentration, reducing it at higher concentrations; TEA showed a concentration-dependent increase of cell proliferation with a MPE of +34% at a 10^{-4}M concentration; NS1619 and ACTZ showed a MPE of +181.6% at 5x10^{-5}M and +135% at 100x10^{-6}M concentration respectively. Staurosporine (STS) (2x10-6M), a broad spectrum protein kinases inhibitor, prevented the IbTX and TEA proliferative action. These results suggest that BK channel may play a role in the regulation of neuronal viability through an intracellular pathway that involves STS-sensitive protein kinases. These findings may have relevance in the cellular repair mechanisms in the neuromuscular disorders. Supported by Telethon GG14096.

2961-Pos Board B391

Long-Term Modulation of Ion Channels by Aldosterone in Adult Rat Atrial Myocytes

Erick B. Rios-Perez, Maricela García-Castañeda, Guillermo Avila.
Cinvestav, IPN, Mexico, Mexico.

In recent years, both aldosterone and mineralocorticoid receptor (MR) have drawn attention as important factors that promote structural remodeling of the atrium. Here, we investigated effects of chronic aldosterone treatment on both intracellular Ca^{2+} and ion channels. Atrial myocytes were cultured in either the absence or presence of aldosterone, and then the activity of ion channels was studied, under whole-cell patch-clamp conditions. Aldosterone increased both the cell membrane capacitance (Cm) and the maximal conductance of ion channels that give rise to IKsus, INa, ICaT, and ICaL (30-100%). Except for inactivation curves of INa, which were shifted by −10 mV, aldosterone produced no major alterations in the biophysical properties of the channels. Interestingly, at resting membrane potentials the increase in INa was cancelled by a greater fraction of inactivation. The onset and recovery of the changes -in ICaT, ICaL, and Cm- were also assessed. In general, they required 2d to be noticeable, reached their maximal value in 6d, and returned to basal values after 1-3d of aldosterone removal. The effects on both Cm and ICaL were further studied -to explore both a potential dose-response relationship and a possible implication of the MR. In fact, co-incubating with 10 μM of spironolactone (an MR antagonist) abolished both effects. Furthermore, their corresponding magnitudes fitted well with the Hill equation, being the EC50 values for Cm and ICaL 20 and 130 (nM), respectively. Interestingly, aldosterone did not alter expression levels of Cav1.2, suggesting that the action on ICaL arises from a stimulus in open probability. The hormone also produced a 40% increase in the amplitude of Ca^{2+} transients along with a higher proportion of arrhythmic cells (2.5-fold increase). These results contribute to understanding the role of the MR and aldosterone in atrial electrophysiology.

2962-Pos Board B392

The Anti-Proliferative Effect of Cation Channel Blockers on T Lymphocytes Stimulated by Anti-CD3 and Anti-CD28

Zoltan Varga, Zoltan Petho, Andras Balajthy, Adam Bartok, Sandor Somodi, Orsolya Szilagyi, Gyorgy Panyi.
Dept. of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary.

The ion channels of T lymphocytes form a crucial part of the healthy immune system, as they are important for cellular activation and proliferation. In